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Petal-specific promoter and method for obtaining plants having flowers with no petals

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(54) Title: PETAL-SPECIFIC PROMOTER AND METHOD FOR OBTAINING PLANTS HAVING FLOWERS WITH NO PETALS

(54) Titre: PROMOTEUR SPECIFIQUE DES PETALES ET PROCEDE D'OBTENTION DE PLANTES A FLEURS SANS PETALE

(57) Abstract

The invention concerns a petal-specific promoter and a method for obtaining plants having flowers with no petals.

(57) Abrégé

L'invention concerne un promoteur spécifique des pétales ainsi qu'un procédé d'obtention de plantes à fleurs sans pétale.

PETAL-SPECIFIC PROMOTER AND METHOD FOR PRODUCING PLANTS HAVING FLOWERS WITH NO PETALS

The present invention concerns, in particular, a petal-specific promoter and a method for producing plants having flowers with no petals.

The advantage of producing plants lacking petals came from the observation that senescent petals, by falling onto the leaves, might provide preferred seats of infection for the spores of certain pathogenic fungi. In the case of rape, for example, the mode of infection of *Sclerotinia sclerotiorum* follows principally this route. This fungus is indeed responsible for important damage in cultures of rape (Lamarque, 1983), and no genetic resistance is known to this fungus, either in rape or in the neighboring species. Thus, at the current time, only preventive chemical treatments are used.

Sclerotinia sclerotiorum control via plants whose flowers would have no petals would make it possible to diminish the use of fungicide, and thus to limit the subsequent pollution of the soils.

It involves, therefore, producing plants having flowers with no petals, and in this way testing a strategy of control of the abovementioned fungus, based on a "physical" resistance and not on the use of resistance genes in the conventional sense.

The present invention proposes, therefore, to produce plants whose flowers would be lacking in petals. It consists in using a promoter region which controls the expression, specifically in the petals, of a sequence (orf) encoding a molecule which is capable of modifying the natural properties of the petal, or of inhibiting the formation thereof.

In this way, modifying the structure, the shape, the coloration and/or the petal structure of flowers may be envisaged, by placing, downstream of the above-described promoter region, genes which are involved in the biosynthesis of pigments, or regulatory genes such as the MYB proteins (Noda et al. 1994). This

type of experiment has already been carried out (Elomma et al., 1996; Gutterson, 1995). However, the promoters used are rather of constitutive type, such as the 35S of CaMV, whereas it would be advantageous to confine the expression of the transgene to the targeted organ. The creation of original ornamental plants may thus, in the context of the present invention, be envisaged.

A subject of the present invention is, therefore, a nucleotide sequence for which it has been demonstrated that the corresponding gene is expressed specifically in the petal, this nucleotide sequence corresponds to SEQ ID No. 5.

Consequently, a subject of the present invention is a nucleotide sequence which corresponds to all or part:

a) of the sequence according to SEQ ID No. 5, or

b) of a sequence which hybridizes to the sequence according to a), or

c) of a sequence which has at least 80% homology with a) or b).

In the context of the present invention, the most valuable part of this nucleotide sequence is the promoter region, which is defined as being the sequence preceding (on the 5'side) the translation start codon (ATG). *Stricto sensu*, this promoter region stretches from nucleotide 1 to nucleotide 3265 (i.e. to the last nucleotide immediately preceding the ATG codon), but, taking into account the restriction sites, this region preferably stretches from nucleotide 1 to nucleotide 3233 (corresponding to the site *Ava*I), and even more preferably from nucleotide 2911 to nucleotide 3233 of SEQ ID No. 5.

This promoter region precedes, therefore, in the natural state, an orf which is expressed specifically in the petals, and when this orf is replaced (by genetic manipulation) by another orf, whose product is a cytotoxic molecule, the latter is

capable of destroying only said petals. The replacement may also be carried out by a gene part which is capable, during its specific expression in the petal, of modifying the properties of origin thereof.

A subject of the present invention is, therefore, also cell-expression vectors comprising a promoter region as described above, placed upstream of a DNA sequence encoding a product which is capable of modifying the structure, the shape, the coloration and/or the petal texture of flowers, and a method for producing ornamental plants, which comprises the insertion into said plants of one of these vectors. The invention also comprises the case where said DNA sequence encodes a cytotoxic product.

Advantageously, the cytotoxic product in question is a ribonuclease. Specifically, when this RNAse is expressed specifically in the petals, it will destroy all the RNAs thereof, as a result of which the petal will not be able to survive. Preferably, the RNAse is barnase, whose corresponding orf has been isolated from *Bacillus amyloliquefasciens* (Hartley RW, 1988).

It involves, therefore, introducing a vector in accordance with the invention into a bacterial strain which is capable of carrying out the transformation of plant cells, such as *Agrobacterium tumefaciens*. This may, in particular, be carried out by the method of infiltration of *Arabidopsis thaliana* plants, described by Bechtold et al., 1993. This technique consists in introducing the bacterium into the cells of the floral scapes, by infiltration under vacuum. The plants are then planted out under glass, and their seeds harvested. About one seed in a thousand gives rise to plants of which all the cells carry the transgene. The transformation of other plants, and in particular of rape, may be carried out through *Agrobacterium tumefaciens* and/or *Agrobacterium rhizogenes*, with the aid of various techniques which are now conventional

(transformation of foliar disks, of hypocotyls, of floral scapes, etc.), combining a phase of coculture of the bacterium with plant tissues, followed by the selection and regeneration of the transformed cells into whole plants. Other transformation techniques do not use this bacterium, but make it possible to transfer the cloned gene directly into cells or tissues (electroporation, particle gun, etc.) and to select and obtain transformed plants (technique reviewed by Siemens and Schieder).

A subject of the present invention is also plant cells transformed with a vector in accordance with the invention, and plants comprising said cells. The subject of the invention is also plants whose flowers have no petals.

As indicated above, the present invention thus makes it possible to produce plants whose flowers have no petals; the method in accordance with the invention comprising the insertion into the plants of a vector as described above and comprising a DNA sequence encoding a cytotoxic product.

In the context of the present invention, it may also be envisaged to produce hybrid plants by crossing two lines whose combined agronomic qualities would be sought. However, in order for the entomophilous pollination to operate optimally, it is necessary for the parents of the hybrid in question to carry petals. Such a cross is, therefore, only possible by means of a two-component system of activation of the toxic gene. The principle of such a system consists in having two lines, each carrying a constituent which has no cytotoxic activity. The specific toxic activity is then restored in the hybrids of these two lines by combination of the two constituents.

A possible example of such a system consists in inactivating the expression product whose control is desired by insertion of at least one stop codon at the start of the corresponding coding sequence, then adding

into the system, in *trans*, a tRNA, termed "suppressor", which will recognize the stop codon(s) and supply the amino acid it is carrying, instead of terminating the translation. The protein will thus be able to be translated in full, and its activity restored. Such a system has already been tried out regarding the sequence encoding the GUS gene into which the amber stop codon was inserted, the suppressor tRNA used being a leucine carrier. In addition, the functionality of such a system of transactivation using a $tRNA^{Leu}$ suppressor has been verified *in planta* in *Arabidopsis thaliana* and *Nicotiana tabacum*. This model was then applied to the case of barnase. Mutated genes (i.e. genes into which a stop codon has been inserted) encoding barnase, and which are dependent upon the expression of the $tRNA^{Leu}$ gene, have been obtained and tested in transient expression in tobacco protoplasts (Choisne Nathalie, 1997).

The present invention thus also concerns a method for producing hybrid plants whose flowers have no petals, and comprising the steps of:

a) transformation of plants of a line A with a vector in accordance with the invention, and comprising a DNA sequence encoding a cytotoxic sequence modified by the insertion of at least one stop codon,

b) crossing of the plants of line A thus obtained with plants of line B expressing the gene of a tRNA suppressor,

c) selection of the hybrid plants having flowers with no petals.

In the context of the present invention, the plants of line A are transformed with a construct similar to pIB352, as represented in Figure 7.

Advantageously, the plants in accordance with the invention belong to the Brassicacea family; preferably, the plant is rape.

Figure 1 illustrates the analysis by Northern hybridization of polyA+ RNA (2 µg) and total RNAs (10 µg) from rape. The membrane is hybridized with the ^{32}P-labeled whole cDNA 9.2. Revelation is carried out after 24 hours of exposure at -80°C with a screen. The mRNAs identified have an approximate size of 800 bp. Plantule 1: plantule of one week; Plantule 2: plantule of two weeks.

Figure 2 illustrates the comparison of the protein sequences from *Arabidopsis thaliana* (above) and from rape (below) deduced, respectively, from cDNA X74360 (SEQ ID No. 1) and 9.2 (SEQ ID No. 2). The protein from *Arabidopsis thaliana* has a length of 140 aa, while the protein from rape has a length of 147 aa, the homology between the two being 74.6%. The stars mark the amino acids which are common to the two sequences, and the dots appearing in the cDNA from *Arabidopsis thaliana* have been indicated only to enable the sequences which are common to the two plants to be placed opposite one another, the *Arabidopsis thaliana* sequence having to be read continuously, i.e. disregarding said dots.

Figure 3 represents the alignment of the nucleotide sequences of the cDNAs 9.2 from rape (below) and X74360 from *Arabidopsis thaliana* (above), the two sequences having a total homology of 83%.

Figure 4 represents the partial restriction maps of the genomic clones (A: *Ava*1, B: *BamH*1, EI: *EcoR*1, EV: *EcoRV*, H: *Hind*III, Hc: *Hinc*II, P: *Pst*I, S: *Sac*1, Sl: *Sal*1, Xb: *Xba*1, Xh: *Xho*1).

Figure 5 represents the 5'→3' sequence of the genomic clone 4.1.1 (SEQ ID No. 5). The palindromic sequence has been underlined twice, the coding sequence has been underlined once. The following restriction sites have been marked: *BamH*I (at position 1): GGATCC; *Sal*I (at position 2911): GTCGAC and *Ava*I (at position 3229): CCCGAG.

Figure 6 represents the constructions carried out with the promoters of the genomic clones 4.1.1 and 8.1.1.

distal promoter region of the genomic clone 4.1.1
palindromic sequence
proximal promoter region of the genomic clone 4.1.1
322 bp promoter region of the genomic clone 4.1.1
322 bp promoter region of the genomic clone 8.1.1
terminator of the nopaline synthase gene
coding sequence of the gus reporter gene
coding sequence of the gene 4.1.1
3' untranslated region of the gene 4.1.1

Figure 7 illustrates the constructs prepared with the 322 bp promoter of the genomic clone 4.1.1.

322 bp promoter of the genomic clone 4.1.1
coding sequence of the gus reporter gene
coding sequence of the gene for wild-type barnase
coding sequence of the gene for mutated barnase
terminator of the nopaline synthase gene
terminator 19S of CaMV

The invention is not limited to sole description above, it will be better understood in the light of the examples below, which are, however, given only as illustrations.

EXAMPLE 1: Demonstration of a petal-specific promoter

The first step consists in obtaining complementary DNA (cDNA) clones which are expressed specifically in the petal. For this, the cDNAs were synthesized from petal messenger RNA (mRNA) from rape. In parallel, cDNAs were synthesized from mRNA from

leaves, from floral buds whose petals have been removed and from stamens.

The cDNAs from said organs or tissues were subtracted from the cDNAs derived from the mRNAs which were expressed in the rape petal. The molecules resulting from this subtraction were used in an experiment of differential hybridization of a petal cDNA library, according to a technique similar to that presented by Atanassov et al., 1996.

Several rape DNA clones were isolated at the conclusion of this experiment. Their expression profile was studied by the technique of Northern molecular hybridization. In the absence of clones which are strictly specific for the petal (at the detection threshold of the technique), the most relevant candidate was retained for the rest of the studies; it is clone 9.2. This clone is strongly expressed in the petal at the young stage (bud of about 3 mm) and very weakly in the stamens (Figure 1).

Homology searches of sequences in the databanks show a strong similarity between the protein deduced from the open reading frame (orf) of clone 9.2 and the coding sequence of an *Arabidopsis thaliana* gene (X74360) which encodes a putative wall protein, whose expression is regulated by the gibberellins (Phillips and Huttly, 1994) (Figure 2). The degree of homology shown by the corresponding respective cDNA sequences is greater than 80% in the first 500 bases, then disappears totally over the remaining 220 (Figure 3).

The rape cDNA clone 9.2 was used as a probe to screen a rape genomic library. Seven genomic clones were isolated. On the basis of the restriction maps and the sequences, these seven clones divide up into two groups, suggesting the existence in rape of a family of at least two genes, named, in the remainder of the text, 4.1.1 and 8.1.1 (Figure 4). The cDNA 9.2 is derived from the gene corresponding to the genomic clone 4.1.1.

A preliminary study by PCR amplification was carried out on the clone 9.4.1 which belongs to the group of 4.1.1. Specifically, the structure of the genomic clone made it possible to amplify an upstream region of 3233 bp, using techniques of amplification of large DNA fragments, and of progressive sequencing by PCR.

This 3233 bp region stretches from nucleotide 1 to nucleotide 3233 of the sequence represented in Figure 5, and it ends at the level of the *AvaI* site, at the level of which the cleavage was carried out, as well as the cloning, to obtain "blunt ends".

Then, the upstream regions possibly containing the regulatory sequences were subcloned from the two genomic clones (4.1.1 and 8.1.1) into cloning vectors. Currently, more than 4 kb of sequence corresponding, in the majority, to the orf and to the upstream regions (Figure 5) are thus available for the clone 4.1.1.

EXAMPLE 2: Verification of the specificity of the promoter region

Different constructs comprising the GUS reporter gene placed under the control of certain of these sequences were prepared in order to study the expression of these chimeric genes (i.e. consisting of the coding sequence of a known gene, preceded by the promoter region in accordance with the invention) in transformed plants from *Arabidopsis thaliana* and from rape.

These constructs fall into two categories, as a function of the orf which is placed under the control of the regulatory sequences:

- the GUS reporter gene, to study the expression profiles and verify the specificity conferred by the promoter,
- the gene for wild-type or inactivated barnase, to prevent the formation of the petal by expression, in this organ, of this

toxic gene (Figures 6 and 7 detail the composition of each construct).

The expression profiles of the GUS reporter gene, in the *Arabidopsis* transformants obtained in the case of the pIB100, show a certain variability over the plants as a whole (see Table 1 below, which enumerates the parts of the transformed plants in which a blue coloration was observed). However, in nearly half the plants having a blue coloration (13/30), the reporter gene is expressed only in the petals (at the detection threshold of the technique). In certain plants, a weak expression in the stamens, which is relatively unsurprising on account of the results of the Northern hybridizations, but also sometimes an expression in other floral organs, is found, which might suggest the influence of positional effects of the transgene, due to its small size. However, the existence of a significant proportion of plants having the expected profile leads to the thought that the 322 bp proximal fragment is capable of conferring an expression which is specific to the petal. The stability of this expression was tested in the descendants on the self-fertilization of these plants. For most, the "petal" specificity was indeed found (data not shown).

Longer promoter sequences were also used via the constructs pIB102 and pIB105, and the transformed plants from *Arabidopsis thaliana* were observed (Table 2 enumerates the parts of the plants which are transformed by pIB102 and have a blue coloration, Table 3 enumerates the parts of the plants which are transformed by pIB105 and have a blue coloration). The petal specificity is not again found in the proportion previously observed, because in almost all cases the reporter gene is effectively expressed in the petal, but also in other organs of the flower.

Similarly, transformed rape plants were obtained with a construct comprising, as a regulatory sequence, the 3233 bp upstream fragment of the gene

4.1.1, which was cloned after PCR amplification. In the nine rape plants which could already be observed, the reporter gene is expressed in the petal, but also in other organs of the flower (data not shown), as is observed in *Arabidopsis* with these large promoter regions.

These results suggest that these fragments are too long, whereas it is thought that the preceding one (322 bp) might be a little short and, therefore, amplify the possible positional effects. The latter, however, gives rise to the most promising results.

The promoters pIB351 and pIB352 (Figure 7), which are analogous to the pIB100, but comprise, respectively, the coding sequence of the gene for wild-type barnase, and this same sequence inactivated by insertion of a stop codon (then named mutated barnase), instead of the coding sequence of the reporter gene, have been introduced into *Arabidopsis thaliana* (results not yet available).

TABLE 1

SEPALS	PETALS (Number)	STAMENS	PISTILS	LEAVES	SILIQUES	OTHERS	TRANSFORMED PLANTS (Number)
-	4	-	-	-	-		13
-	4	-	top, stigma	-	-		1
-	4	-	below papilla	-	-		1
-	2/4	-	except papilla	2 tips	-		1
-	1/4 1 flower	-	below papilla, 1 flower	-	-		1
-	4	tip, young	-	-	-		1
-	4	stamen	pistil	-	-	floral peduncle	1
-	4	top, filament	below papilla		-		1
-	4 light	small bud	interior except papilla	-	interior		1
-	4	young	except papilla	-	-		1
bud	4	-	relatively low, stigma	-	-		3
bud	4	connective tissue	stigma	tip	-	floral peduncle	1
tip	4	top, filament	interior	-	-		1
certain	4	connective	pistil	tip + margin	-		1
edge	4	tip	below papilla	-	-		1
edge	4	top, pollen sack	top, stigma	-	-		1
							30 plants

TABLE 2

SEPALS	PETALS (Number)	STAMENS	PISTILS	LEAVES	SILIQUE	OTHERS	TRANSFORMED PLANTS (Number)
-	2 of a few flowers	-	-	-	-		1
-	4	-	below papilla	-	-		6
-	4	filament	below papilla	-	-		7
bud	4	-	below papilla	-	-		1
bud	4	pollen sack; filament	below papilla	-	-		2
+	+	+	except papilla	-	-		1
bud	4	entire bud	except old papilla	tip, top	-	floral peduncle	1
							19 plants

TABLE 3

SEPAL	PETAL (Number)	STAMEN	PISTIL	LEAF	SILIQUE	OTHERS	TRANSFORMED PLANTS (Number)
-	1 flower	-	-	-	-		1
-	-	-	below papilla	-	-		1
-	4	pollen sack, filament	below papilla	-	-		6
-	4	entire	except papilla	border	-	floral peduncle	1
bud	-	-	-	-	-		1
bud	4	entire	except papilla	-	-		1
bud	4	entire	except papilla	small	-	floral peduncle	3
bud	4	pollen sack, filament	below papilla	-	-		19
bud	4	filament	below papilla	tips	-		3
							36 plants

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EDITORIAL NOTE FOR APPLICATION

NO. 92708/98

THE FOLLOWING SEQUENCE LISTING, WITH PAGE NO.'S 1 - 7, IS PART OF THE DESCRIPTION

THE CLAIMS BEGIN DIRECTLY AFTER THAT ON PAGE NO. 16

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(B) STREET: 147 RUE DE l'UNIVERSITE
(C) CITY: PARIS
(E) COUNTRY: FRANCE
(F) POSTAL CODE: 75007

(ii) TITLE OF THE INVENTION: PETAL-SPECIFIC PROMOTER AND METHOD FOR PRODUCING PLANTS HAVING FLOWERS WITH NO PETALS

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: A. thaliana protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Ala Ser Ser Leu Ile Thr Ser Ala Val Ile Val Val Val Leu Ser
1               5                  10                  15

Leu Val Leu Gly Ser Val Glu Gln Val Ser Gly Leu Arg His Val Pro
            20                  25                  30

Lys Ser Pro Lys Ile Thr Asp Val Lys His Pro Asp Phe Leu Val Thr
        35                  40                  45

Ile Glu Pro Lys Pro Thr Ile Leu Ile Pro Gly Val Gly Arg Phe Leu
    50                  55                  60
```

Leu Pro Pro Lys Cys Lys Lys Pro Phe Tyr Pro Tyr Asn Pro Val Thr
65 70 75 80

Gly Ala Pro Leu Thr Gly Gly Gly Ile Pro Ser Tyr Asn Gly Gly Gln
85 90 95

Gly Ala Gly Pro His Thr Gln Leu Pro Gly Gly Asp Asp Thr Leu Val
100 105 110

Pro Asn Pro Gly Phe Glu Glu Pro Thr Pro Thr Ile Gly Ala Gly Thr
115 120 125

Gly Ser Asn Gly Gln Val Pro Pro Val Pro Leu Pro
130 135 140

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: rape protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ala Ser Ser Leu Leu Thr Leu Ala Ala Ala Ala Val Thr Val Met
1 5 10 15

Ile Leu Ser Leu Leu Leu Gly Pro Ala Glu Gln Val Ser Gly Leu Arg
20 25 30

His Ile Pro Lys Ser His Lys Thr Thr Asp Val Lys His Pro Glu Phe
35 40 45

Leu Val Thr Ile Glu Pro Lys Pro Thr Ile Leu Ile Pro Gly Val Gly
50 55 60

Arg Phe Leu Leu Pro Pro Lys Cys Lys Lys Pro Phe Tyr Pro Tyr Asn
65 70 75 80

Pro Val Thr Gly Ala Pro Leu Thr Gly Gly Ser Ile Gly Gly Gln Ile
85 90 95

Pro Ser Phe Gly Gly Gly Gln Gly Gly Gly Ala Arg Thr Gln Leu Pro
100 105 110

Gly Gly Asp Asp Thr Leu Val Pro Asn Pro Gly Phe Glu Thr Pro Thr
115 120 125

Pro Ala Thr Gly Ala Gly Ala Gly Asn Asn Gly Gln Val Pro Pro Val
130 135 140

Pro Leu Pro
145

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 641 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: clone 9.2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AGCAGTCACT GTCATGATTC TTAGCCTACT GCTTGGACCT GCAGAGCAAG TTAGCGGACT 60

GCGTCATATT CCCAAGTCCC ATAAGACCAC TGATGTCAAA CACCCTGAGT TTCTTGTCAC 120

CATTGAGCCA AAACCAACTA TTCTCATCCC CGGTGTTGGA AGGTTCTTGC TTCCTCCCAA 180

ATGTAAGAAA CCATTCTACC CATACAATCC AGTCACTGGA GCTCCCCTTA CTGGCGGGTC 240

TATCGGTGGT CAAATCCCAT CATTTGGTGG TGGACAAGGA GGCGGAGCTC GCACCCAGCT 300

CCCTGGTGGC GATGATACCC TTGTCCCAAA CCCCGGATTT GAAACTCCAA CCCCTGCCAC 360

TGGAGCTGGC GCTGGAAACA ACGGCCAAGT TCCTCCGGTG CCACTACCCT GATTTCTTTT 420

TCAATATCTG TCAACAAATA AGCATTTCTT TAATGCAAAA GTGTCTATTT GAGTCTTACC 480

TTCTGGTTTA CTAGCCGTCA CCTTAAGAGT CATATGTTTG TCATCTCTCT CTTTCTTTTT 540

GGAAGAGAGA ATCTTGTGTC TTATGCCGTC AGAAGAAATT TAAAGCATTT GTTTACATGC 600

CATTACATTC AACTATCAAA ATGCTTTATG ATAAAAAAAA A 641

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 711 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: X74360

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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TCACGTTCCC AAGTCCCCTA AGATCACTGA TGTCAAACAC CCTGACTTTC TTGTAACCAT 180

TGAGCCCAAA CCAACTATTC TCATTCCCGG TGTTGGAAGG TTCTTGCTTC CTCCCAAATG 240

CAAGAAGCCG TTCTACCCTT ACAATCCTGT CACCGGAGCT CCACTTACTG GTGGGGGAAT 300

CCCATCATAT AATGGTGGAC AAGGGGCCGG ACCTCACACC CAACTCCCTG GTGGCGATGA 360

TACGCTTGTC CCAAACCCCG GATTTGAAGA GCCAACCCCG ACCATTGGAG CTGGCACAGG 420

AAGCAACGGC CAAGTTCCAC CAGTGCCACT ACCCTGAGTA TTATTAATCT GTCAACAAAT 480

AAGCATATCT TAGATGCAAA CATGTCTGTT TTGGTGTCTT GAGTCTTGGT TAGATAAGTA 540

ACCCGCTACT TTACTAGCCG TTTCGTTTGC CATCTCTTTT TCTCTCTGTG TCTCTCTCTA 600

TTTGCTACAA AAAGAGAGAA TCTTGTTTCA TGTTTTTCAG TTTGTCTTTA GATGAATTCA 660

TTTTCACATA CCATTATATT AAAATAAAGG AAATGTTCCG CAGTAAAAAA A 711

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4516 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: genomic clone 4.1.1

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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AATTTCCNGA ACTAAAAACA GANNAGANAN NAGGCTTACC ATTTCCATGC CNAGANGANG 4500

GTATCTCTCC AAAGCC 4516

CLAIMS

1. Nucleotide sequence corresponding to all or part:

   a) of the sequence according to SEQ ID No. 5, or

   b) of a sequence which hybridizes to the sequence according to a).

2. Nucleotide sequence according to Claim 1, corresponding to all or part:

   a) of the sequence which stretches from nucleotide 1 to nucleotide 3233 and preferably from nucleotide 2911 to nucleotide 3233 of SEQ ID No. 5, or

   b) of a sequence which hybridizes to the sequence according to a), or

   c) of a sequence which has at least 80% homology with a) or b).

3. Cell-expression vector comprising a sequence according to Claim 2, placed upstream of a DNA sequence encoding a product which is capable of modifying the structure, the shape, the coloration and/or the petal texture of flowers.

4. Cell-expression vector comprising a sequence according to Claim 2, placed upstream of a DNA sequence encoding a cytotoxic product.

5. Vector according to Claim 4, characterized in that the cytotoxic product is a ribonuclease and preferably barnase.

6. Plant cells transformed with a vector according to one of Claims 3 to 5.

7. Plants comprising cells according to Claim 6.

8. Method for producing ornamental plants, comprising the insertion into said plants of a vector according to Claim 3.

9. Method for producing plants whose flowers have no petals, comprising the insertion into said plants of a vector according to Claim 4 or 5.

10. Method for producing hybrid plants whose flowers have no petals, comprising the steps of:

a) transformation of plants of a line A with a vector according to Claim 4 or 5, modified by insertion of at least one stop codon into the coding sequence of the DNA,

b) crossing of the plants of line A obtained in a) with plants of line B expressing the gene of a tRNA suppressor,

c) selection of the hybrid plants having flowers with no petals.

11. Plants whose flowers have no petals, and which are capable of being produced by implementing the method according to Claim 9 or 10.

12. Plants according to Claim 7 or obtained by the use of the method according to Claim 9 or 10, characterized in that they belong to the Brassicacea family, preferably in that they are rape.

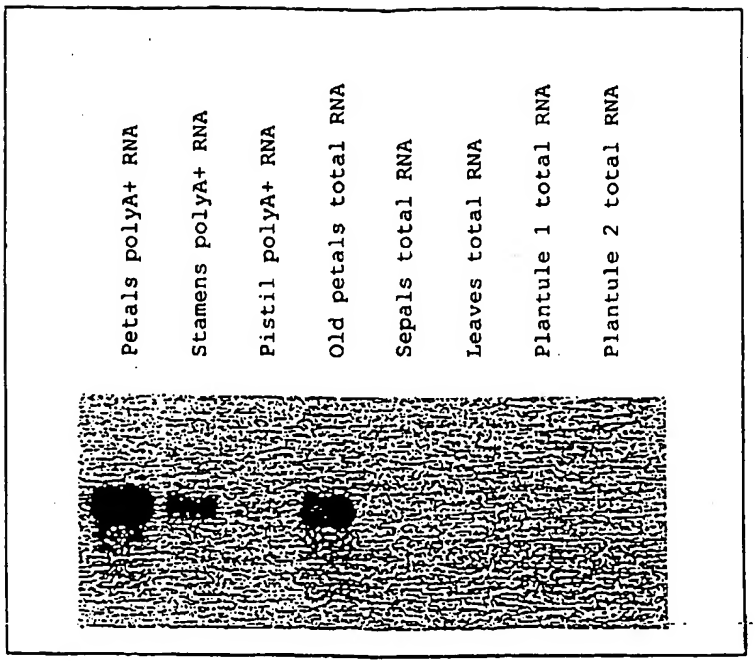
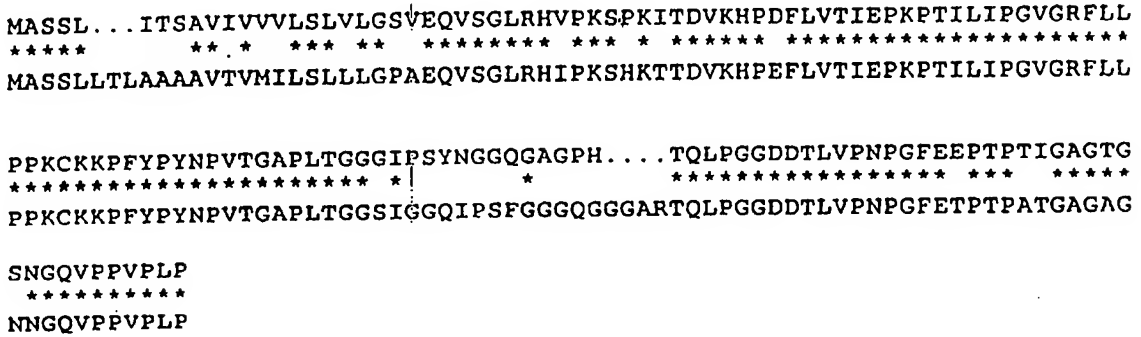

Petals polyA+ RNA
Stamens polyA+ RNA
Pistil polyA+ RNA
Old petals total RNA
Sepals total RNA
Leaves total RNA
Plantule 1 total RNA
Plantule 2 total RNA

FIGURE 1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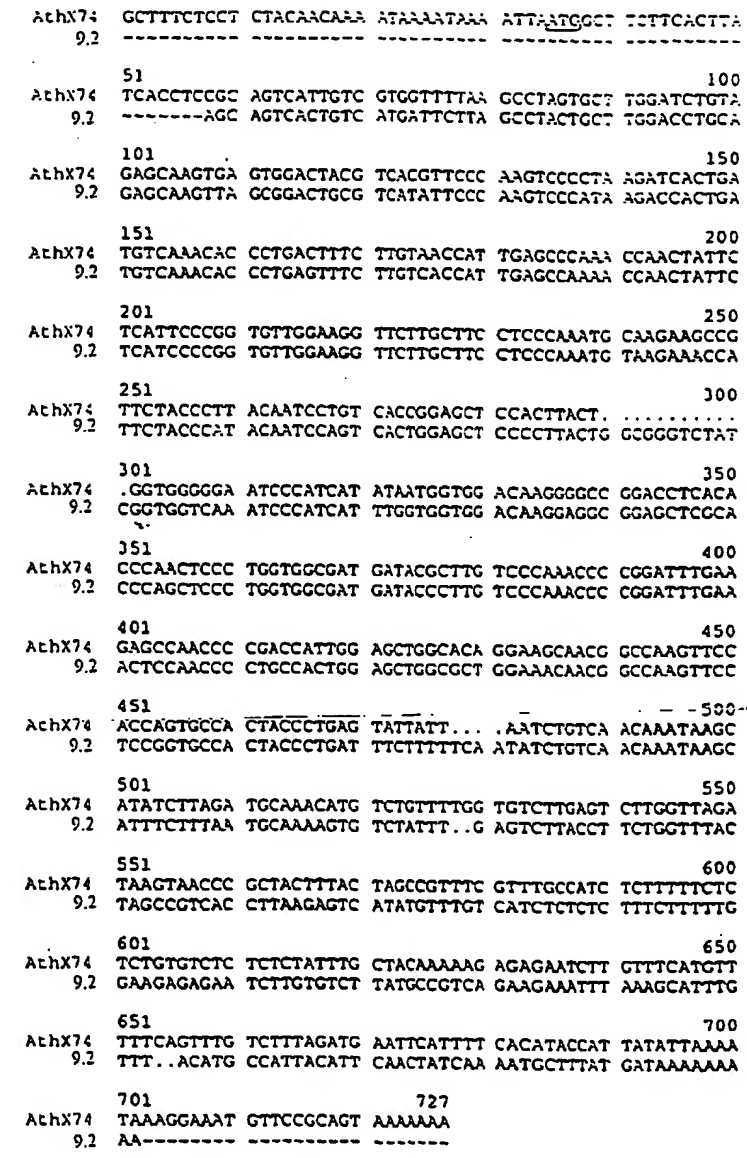
NNGQVPPVPLP

FIGURE 2

FIGURE 3



Nucleotide alignment of the cDNAs X74360 and 9.2

FIGURE 4

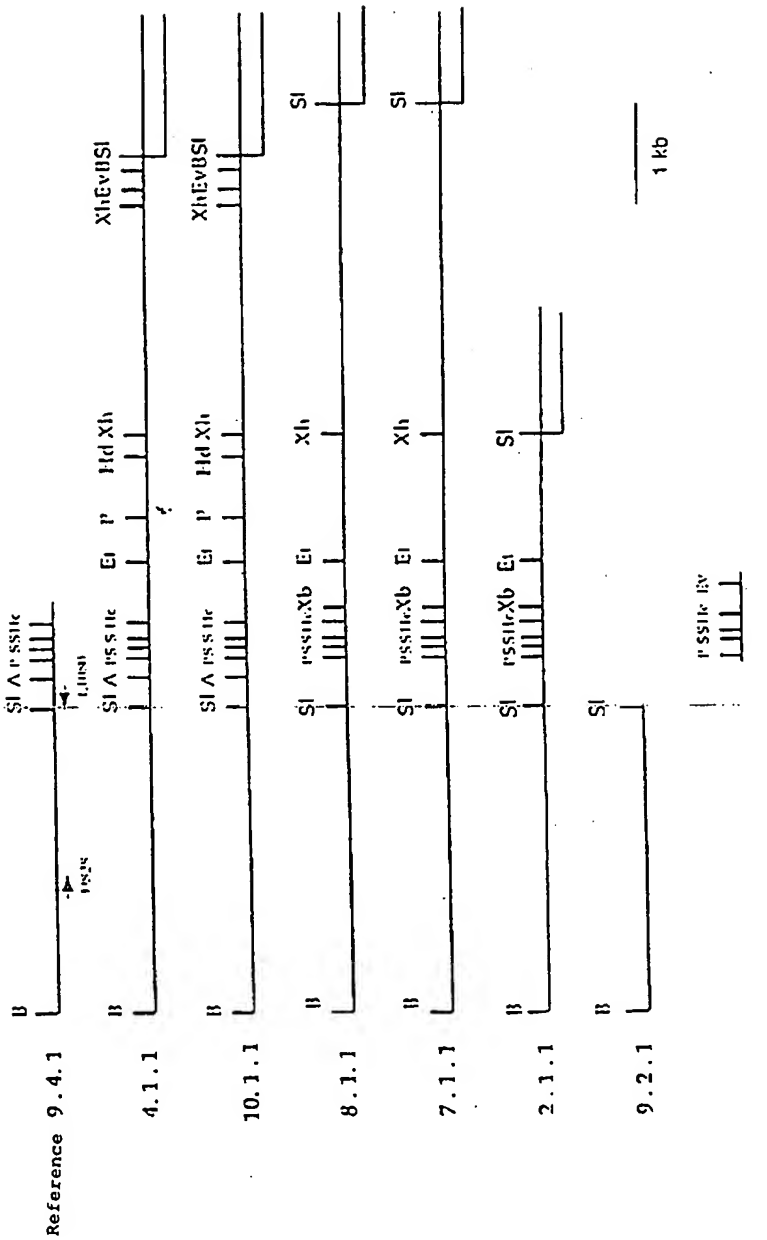
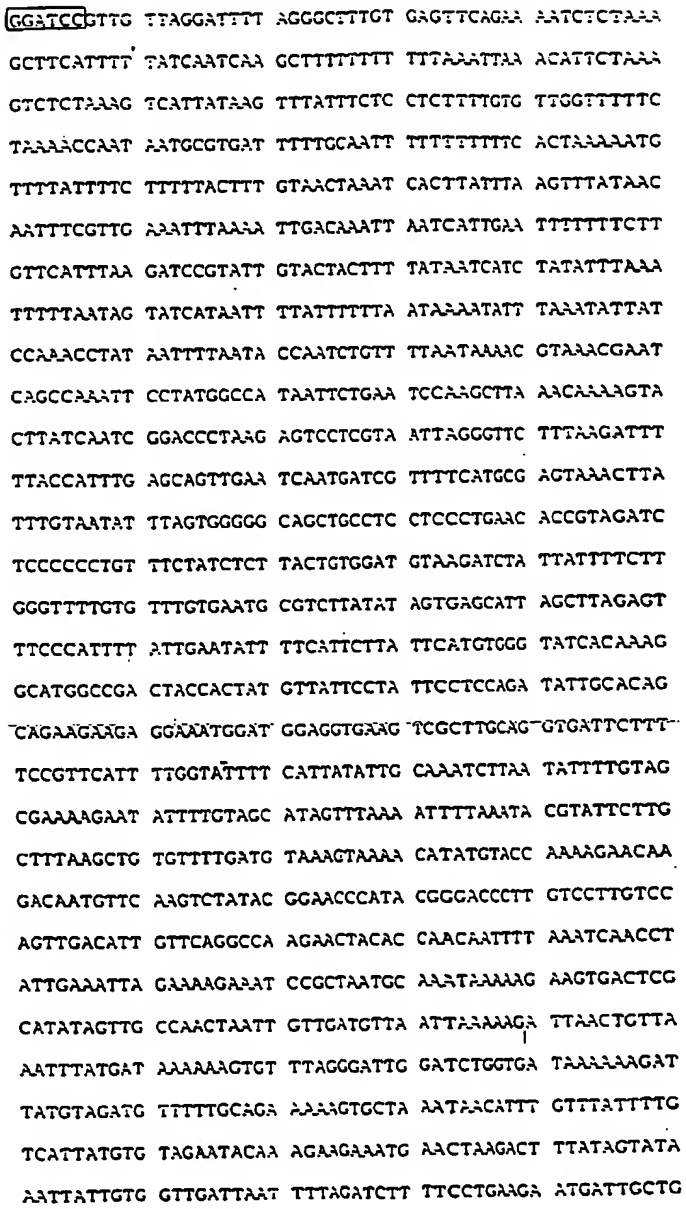


FIGURE 5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101 GTCTCTAAAG TCATTATAAG TTTATTTCTC CTCTTTTGTG TTGGTTTTTC
151 TAAAACCAAT AATGCGTGAT TTTTGCAATT TTTTTTTTTC ACTAAAAATG
201 TTTTATTTTC TTTTTACTTT GTAACTAAAT CACTTATTTA AGTTTATAAC
251 AATTTCGTTG AAATTTAAAA TTGACAAATT AATCATTGAA TTTTTTTCTT
301 GTTCATTTAA GATCCGTATT GTACTACTTT TATAATCATC TATATTTAAA
351 TTTTTAATAG TATCATAATT TTATTTTTTA ATAAAATATT TAAATATTAT
401 CCAAACCTAT AATTTTAATA CCAATCTGTT TTAATAAAAC GTAAACGAAT
451 CAGCCAAATT CCTATGGCCA TAATTCTGAA TCCAAGCTTA AACAAAAGTA
501 CTTATCAATC GGACCCTAAG AGTCCTCGTA ATTAGGGTTC TTTAAGATTT
551 TTACCATTTG AGCAGTTGAA TCAATGATCG TTTTCATGCG AGTAAACTTA
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651 TCCCCCCTGT TTCTATCTCT TACTGTGGAT GTAAGATCTA TTATTTTCTT
701 GGGTTTTGTG TTTGTGAATG CGTCTTATAT AGTGAGCATT AGCTTAGAGT
751 TTCCCATTTT ATTGAATATT TTCATTCTTA TTCATGTGGG TATCACAAAG
801 GCATGGCCGA CTACCACTAT GTTATTCCTA TTCCTCCAGA TATTGCACAG
851 CAGAAGAAGA GGAAATGGAT GGAGGTGAAG TCGCTTGCAG GTGATTCTTT
901 TCCGTTCATT TTGGTATTTT CATTATATTG CAAATCTTAA TATTTTGTAG
951 CGAAAAGAAT ATTTTGTAGC ATAGTTTAAA ATTTTAAATA CGTATTCTTG
1001 CTTTAAGCTG TGTTTTGATG TAAAGTAAAA CATATGTACC AAAAGAACAA
1051 GACAATGTTC AAGTCTATAC GGAACCCATA CGGGACCCTT GTCCTTGTCC
1101 AGTTGACATT GTTCAGGCCA AGAACTACAC CAACAATTTT AAATCAACCT
1151 ATTGAAATTA GAAAAGAAAT CCGCTAATGC AAATAAAAAG AAGTGACTCG
1201 CATATAGTTG CCAACTAATT GTTGATGTTA ATTAAAAAGA TTAACTGTTA
1251 AATTTATGAT AAAAAAGTGT TTAGGGATTG GATCTGGTGA TAAAAAAGAT
1301 TATGTAGATG TTTTTGCAGA AAAAGTGCTA AATAACATTT GTTTATTTTG
1351 TCATTATGTG TAGAATACAA AGAAGAAATG AACTAAGACT TTATAGTATA
1401 AATTATTGTG GTTGATTAAT TTTAGATCTT TTCCTGAAGA ATGATTGCTG

FIGURE 5 (continued)

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1451  AATAATAAAA TGTTCATTTG CTTAATGAGT ATGTCTACTC TTTAGTTATT
1501  TCTGACCCGA AACCAACAAA CACTAATGAT TGATTAAACT AACCAATCAA
1551  CTTAACTTGT AAAACGAGTT GGCTTAGAAC ATGATTATTG AGAGGTTCTT
1601  AGGGTGGAGT TCTTAGCGGA ATATAAGAAC CTGTGTCTTA ATTTTTAATT
1651  AAAAAAGCTA AGAACTGGCT CTTAAATAAG AGTTTAAGAG CCGGTTCTTA
1701  GTTTTTTTAG TTAAAAGTTA AGAGTCAGGT TTTTATATTC CGTTAAGAAC
1751  TTCACCTTAA GGACCTTCTA ATAATCATGC TCTTACGTTA TCTGACCAAA
1801  AATACGAACA GAAAAAATAA AAACTCACTT ACCTCATCAT ATGAGATATG
1851  ACAAATGCAC TACTATTTAA GAAAAAACAT TAAAAAAAAC ATTAATGGTG
1901  TGGGAGGGTC ATTAATGGAG GTCACACAAA AGAAAGGCCA GAGAAGGCAA
1951  ATTGAAGGTG ACTGTATACA AAAGTAGGTC TTTCAGTTTT GCNCAGAGGA
2001  AGCTCATGAC ATTCACCAAA GCAGCACGAA TGAAGTTCAT CAAGTTTTTA
2051  ATTAGGCTTC GCTTCTTGTG ATTCCTCGAA AATTTATATC ATTTCATACG
2101  TTCGTTCTTG TTTTCATGTG ACTTTCCTCT TCTCCTACCG TGAGTCTCAT
2151  CAATTTCGTA GATCGCTANG TTAACGATCC ACGTATCATA NATACACTTC
2201  TTTCTATAGC CGTACGTATA CCACACATTA CNTCATCCCA CTTCNTAACT
2251  TATATAATTT TACTACTCAG ATCACNAGAG TACGTATATC AGGAAGTCAT
2301  TTCTTCTCCT TGTCCTATTC CTCTCTTTCT TTGTCCGGGT CTTATGTTCG
2351  CTAGTAGGAA TTTTCCGACG CACCCTTATC CAAGTATGTA TGCTATTCTC
2401  TCTCACTCTC CTTAATTTTA CACACCTCTT TCACTATCTT CAATGTCTTT
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2601  ATGCTCTCCA TGCCGTCTTG TGATGGTTAG CTTCGTGTGT GCATCGCTAG
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2701  TCCTACCCAG TCCCATGATG AATTAGCCTC TCTCACACTT AACTCTATGC
2751  ATTCAGACGT TTTGTTTCTT TCCTTTTGCT TCTTCGGATA AATTACCCTG
2801  TGTATGTATA AAATGCATCT TTTCCTTTTT TTAATTCTTT TGTCTTTTTG
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FIGURE 5 (continued)

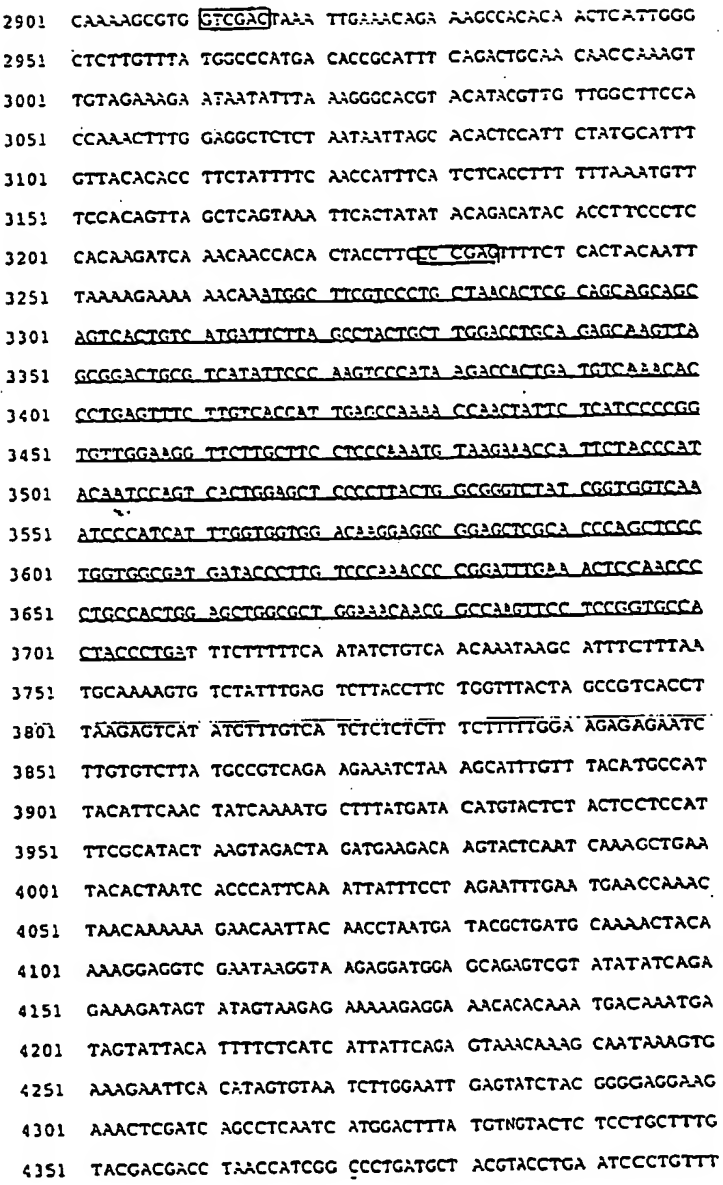

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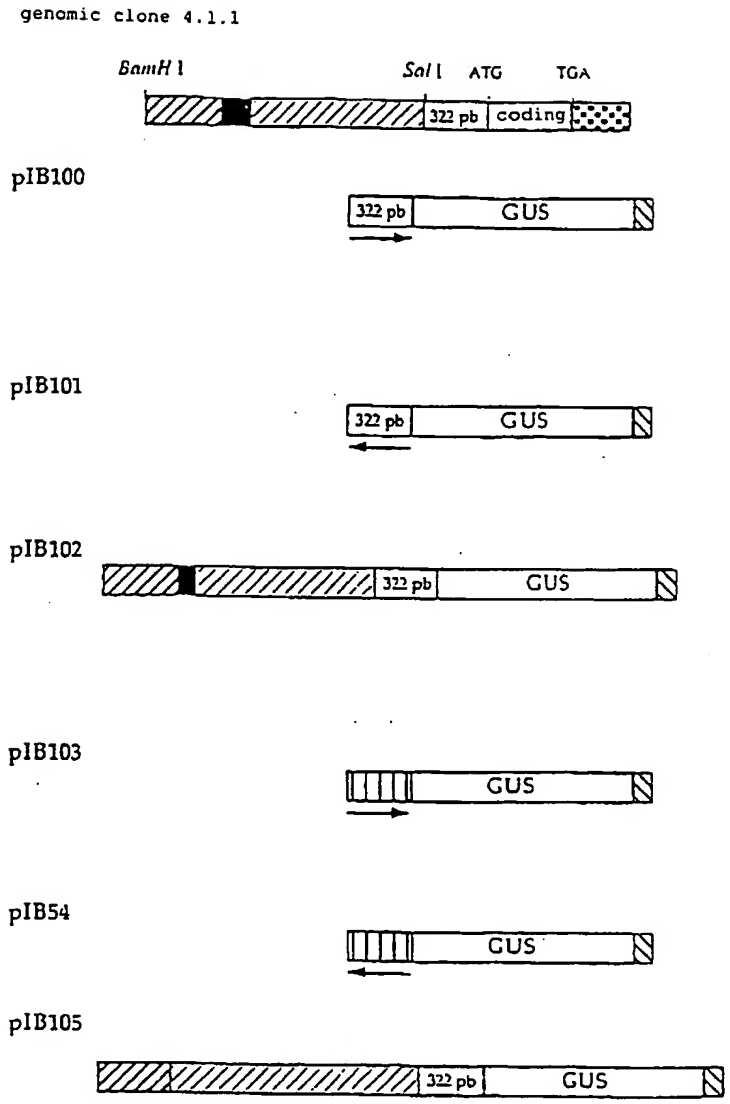
FIGURE 5 (continued)

FIGURE 6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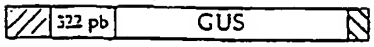

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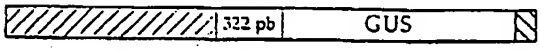

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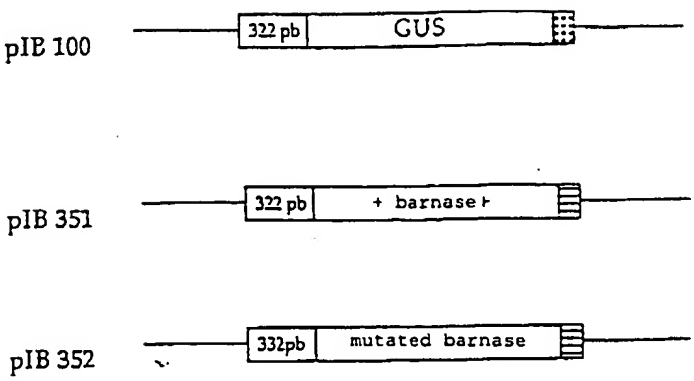
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FIGURE 7